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Exploring Bioactive Compound Marine Sponge *Achantella* sp. as Antibacterial Agent from Pahawang Island Water, Lampung

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ABSTRACT

Indonesia has a huge potential for marine biological resources. Sponges are one of those marine resources. Sponges produce bioactive compounds with diverse chemical structures. Many studies report the potential of marine sponge extracts as natural antibacterial agents. One of a sponge widely found in Indonesian waters is *Achantella* sp. The purpose of this study was to analyze the extract of the sponge *Achantella* sp. as an antibacterial agent against pathogenic bacteria. Yield of *Acanthella* sp. extract from the extraction used methanol solvents 3.22%, ethyl acetate 1.53%, and hexane 0.93%. The *Acanthella* sp. extracts demonstrated antibacterial activity against the pathogenic bacterium *E. coli*, where the methanol, ethyl acetate, and hexane extracts produced inhibition zones of 5.59 mm, 7.60 mm, and 5.90 mm.

INTRODUCTION

Indonesia has a huge potential for marine biological resources. Sponges are one of those marine resources. Sponges are invertebrate animals from the Phylum Porifera that can be found in tropical and subtropical waters. Sponges are primitive, sessile invertebrates that live on the aquatic floor. Sponges do not move but stay and live in one place until adulthood, just like plants. Sponges belong to the Phylum Porifera, which divides into three classes: Calcarea, Hexactinellida, and Demospongiae. The class Calcarea, for example, possesses a simpler structure compared to the others, and its spicules consist of calcium carbonate in the form of calcite. The class Hexactinellida represents glass sponges, whose spicules consist of silicates and lack spongin, which chemically relates with silk. The class Demospongiae has a massive shape and bright colors, featuring a complex canal system that connects to small, rounded flagellated chambers. Some of their spicules consist of silicates, while others contain spongin (Putra *et al.*, 2024).

Sponges have three main cellular layers. The first layer is the pinacoderm, which sits on the outer surface of the sponge and comprises a single layer of cells that scientists call pinacocytes. The second layer is the choanoderm, which features choanocytes that possess collar cells. Choanocytes function to create currents and direct water through the canal system of the sponge. The third layer is the mesohyl. This layer forms a protein matrix that sits

between the pinacoderm and the choanoderm, where researchers find skeletal material alongside all other cell types. Within the mesohyl, amoebocytes move from one choanocyte to another to collect nutrients from these cells and then distribute them to other cells. Additionally, amoebocytes actively ingest and excrete skeletal elements, specifically spicules (Pan *et al*., 2026).

Sponges are an example of porous invertebrate animals shaped like tubes or vases. These marine organisms produce various secondary metabolite compounds with antibacterial activity. Sponges produce bioactive compounds with diverse chemical structures. Many studies report the potential of marine sponge extracts as natural antibacterial agents. One of a sponge widely found in Indonesian waters is *Achantella* sp. *Achantella* sp. produces many secondary metabolites (Abdel-Razek *et al*., 2023). Jiao *et al*. (2022) isolate a new terpenoid compound, namely kalihioxepane, from this sponge. This sponge is very benefit because it has antioxidant and cytotoxic activities. This sponge potentially serves as an antibacterial agent (Fakhri *et al*., 2013). Previous studies show that the sponge *Achantella* sp. possesses potential as a source of antioxidants (Putra *et al*., 2016; Shen *et al*., 2022; Ibrahim *et al*., 2023) and antibacterial compounds (Yanti *et al*., 2015). Sponges contain terpenoid bioactive compounds as antibacterial agents (Wang *et al*., 2022). In addition to sponge extracts, extracts from sponge symbiont microbes also offer potential as effective natural antibacterial agents (Murniasih, 2016). The purpose of this study was to analyze the extract of the sponge *Achantella* sp. as an antibacterial agent against pathogenic bacteria.

METHODS

Time and Location

This research conducted this study from May to November 2025. The sponge samples were collected from the waters of Pahawang Island, Pesawaran Regency. The sponge samples were analyzed at the Oceanography Laboratory, University of Lampung.

Materials and Equipment

The equipment used in this study included SCUBA (Self-Contained Underwater Breathing Apparatus) diving gear, masks, gloves, scissors, 600 ml bottles, a cool box, rulers, knives, Erlenmeyer flasks, a rotary evaporator, an analytical balance, separating funnels, graduated cylinders, petri dishes, an autoclave, tweezers, spirit burners, a magnetic stirrer, dropping pipettes, microtubes, a hot plate, a vortex mixer, spatulas, a laminar air flow cabinet, test tubes, an incubator, and an underwater camera, while the materials used consisted of sponge identification books, sponge samples, pathogenic bacteria, aquades, methanol, ethyl acetate, hexane, chloramphenicol, disc papers, filter papers, and tissues.

Research Procedure

Sampling Procedure

The sponge samples were collected using SCUBA diving at a depth of 7–10 m. The sponge samples were carefully cut using a diving knife. The researchers documented the shape, size, and color of the sponges in the field. The sponge samples were placed into a cool box for further analysis in the laboratory.

Extraction

The sponge samples were cleaned with running water. The sponge samples were cut into small pieces. The sponge samples were weighed at 50 g of wet weight. The sponge sample was put into a glass container in wet weight. The sponge sample was macerated in three different solvents separately. The active compounds were efficiently extracted into the

solvents by the maceration process. The result from maceration process was filtered through filter paper. The filtrate was evaporated using a rotary vacuum evaporator at 45°C. The resulti extract paste was placed into a vial bottle for storage. The weight of the extract was measured to determine the yield percentage (Kusuma *et al*., 2026).

Negative and Positive Control

The laboratory apparatus and materials were sterilized in an autoclave at 121°C for 15 minutes. Aqua distilled was used as a negative control. Chloramphenicol at a concentration of 0.05% was used as the positive control. Chloramphenicol solids with a dose of 250 mg were crushed and pulverized into a fine powder. The chloramphenicol powder 10 mg was weighed and dissolved in 10 mL of DMSO solvent to obtain a stock solution with a concentration of 1,000 ppm. The stock solution was diluted gradually using aqua distilled. A chloramphenicol solution with a final concentration of 0.05% was yielded by the dilution process. The paper discs were soaked in the solution and utilized as the positive control.

Media Preparation

A total of 12 g of Tryptic Soy Agar (TSA) powder was weighed and dissolved in 300 mL of distilled water in an Erlenmeyer flask, then heated and homogenized using a hotplate and magnetic stirrer. The medium was sterilized in an autoclave, cooled to approximately 45°C, and poured into sterile petri dishes. Once solidified, the Petri dishes were sealed, wrapped in plastic, and incubated at 37°C for 24 hours.

Subculturing of Pathogenic Bacterial

The pathogenic *Escherichia coli* bacteria were streaked onto fresh agar media using a sterile inoculating loop. The pathogenic bacteria were inoculated into a sterile nutrient broth. The cultures were incubated at 37°C for 24 hours to obtain fresh bacterial cells.

Preparation of Bacterial Suspension

A loopful of the rejuvenated bacterial culture was collected using a sterile inoculating loop. The bacterial colony was suspended in 10 mL of a sterile 0.9% NaCl solution. The bacterial cells were inoculated into a sterile saline solution and homogenized. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard. The optical density of the suspension was measured using a spectrophotometer to achieve a concentration of 10^8 CFU/mL. The bacterial suspension was transferred onto the TSA medium using a micropipette. The bacterial suspension was spread in a zig-zag pattern across the entire surface of the medium (Kusuma *et al*., 2023).

Application of Paper Discs

The paper discs were picked up using sterile tweezers. The paper discs were placed onto the surface of the inoculated agar medium. Each disc was gently pressed using sterile forceps to ensure complete contact with the agar surface. The paper discs were arranged equidistant from each other on the petri dish. The Petri dishes were tightly wrapped in paper and incubated at 37°C for 24 hours.

Inhibition Zone Test

The formation of inhibition zones around the paper discs was observed after the incubation period. The diameter of the zone of inhibition was measured using a digital vernier caliper. The clear zones around the discs were measured in mm using a ruler. The inhibition zone measurements were performed in triplicate, and the average values were calculated (Kusuma *et al*., 2023).

Data Analysis

The yield data were analyzed through mathematical calculation by comparing the weight of the crude extract with the dry weight of the raw material. The data analysis of

antibacterial activity was performed by calculating the inhibition zone diameter around the paper discs (Kusuma *et al*., 2026).

RESULTS

Identification of Sponge

The discovered *Acantella* sp. was characterized by a red, shrub-like branched body shape with a prickly and rough surface texture. The sponge *Acanthella* sp. was characterized by a stone-like hard surface and a compressible yet firm body, which was supported internally by a dense skeleton of straight, pointed spicules. The entire body was covered with minute pores for filtering food and water. Additionally, the large water discharge channels (oscules) were small and barely visible to the naked eye (Nascimento et al., 2019; Mangurana et al., 2025; Díaz et al., 2023).



Figure 1. Marine Sponge *Achantella* sp.

Yield of Sponge Extract

Varying values were observed in the extraction yields across the methanol, ethyl acetate, and hexane solvents. The extraction yields of the sponge from the research location are presented in Table 1.

Table 1. Extraction Yields of The Sponge Extracts

Test	Solvent	Weight of Sample (g)	Weight of Yiled (g)	Yield (%)	Yield Average (%)
1	Methanol	50.52	1.63	3.44	3.22
2	Methanol	51.43	1.50	3.00	
1	Ethyl Acetate	50.17	0.77	1.45	1.53
2	Ethyl Acetate	51.20	0.66	1.61	
1	Hexane	50.02	0.47	0.80	0.93
2	Hexane	51.33	0.53	1.06	

Yield of *Acanthella* sp. sponge extract from the extraction used methanol solvents 3.22%, ethyl acetate 1.53%, and hexane 0.93%. The yields of *Acanthella* sp. sponge extract obtained from the extraction were 1.22% for methanol (Zhang *et al*., 2012), 0.50–1.50% for ethyl acetate (Wang *et al*., 2022), and 0.47% for hexane (Xu *et al*., 2012).

Antibacterial Activity of Sponge Extracts

The bioactive compounds demonstrated antibacterial activity by forming a clear zone of inhibition around the paper discs. The antibacterial activity of the sponge extracts is presented in Table 2.

Table 2. Antibacterial Activity of The Sponge Extracts

Test	Inhibition Zone (mm)				
	Methanol	**Ethyl Acetate**	**Hexane**	**K+**	**K-**
1	6.20	6.80	5.70	25.80	-
2	4.80	8.40	6.10	27.50	-
Average	5.50	7.60	5.90	26.60	-

The *Acanthella* sp. extracts demonstrated antibacterial activity against the pathogenic bacterium *E. coli*, where the methanol, ethyl acetate, and hexane extracts produced inhibition zones of 5.59 mm, 7.60 mm, and 5.90 mm. *Acanthella* sp. extracts produced inhibition zones against the pathogenic bacterium *E. coli* ranging from 9.0–23.3 mm for methanol (Ibrahim *et al*., 2023), 14.0–15.0 mm for ethyl acetate (Murniasih *et al*., 2016), and 5.0–9.5 mm for hexane (Ibrahim *et al*., 2023).

DISCUSSION

Acanthella sp. was a genus of marine sponges in coral reef waters. This sponge generally possessed bright orange and red colors in its habitat. This sponge had a porous and branched body on the seabed. The shape of this sponge resembled a small bush. *Acanthella sp.* grew firmly attached to rocks and reef walls. This sponge sometimes inhabited the interior of underwater caves. Common name this sponge was the cactus sponge because its surface featured dense short protrusions. A spicule skeleton made of silica supported the sponge's flexible body texture (Mangurana *et al*. 2025).

The methanol solvent produced a higher extract yield than ethyl acetate and hexane through the maceration method. The *Acanthella sp.* contained many polar secondary metabolite components. Methanol extracted almost all components from the sponge cell matrix because of its polar properties. Methanol molecules easily permeated the sponge cell membrane because of their small size. The universal property of methanol enhanced the effectiveness of solute mass extraction. This polar solvent also extracted high-molecular weight inactive substances, whereas ethyl acetate and hexane bound specific compounds more selectively (Beesoo *et al*., 2021; Miralrio and Vázquez, 2020).

A high yield did not guarantee a high strength of antibacterial activity. The antibacterial activity of the ethyl acetate extract against pathogenic bacteria demonstrated this by being higher than that of methanol and hexane. This was because the methanol solvent diluted the bioactive compound concentration as a result of extracting inactive compounds. Meanwhile, the ethyl acetate solvent dissolved components containing purer and more concentrated active compounds. These concentrated bioactive compounds were able to penetrate the lipid layer of the bacterial cell membrane. The semi-polar solvent bound antimicrobial compounds, especially terpenoids, exceptionally well. Components within the ethyl acetate extract worked synergistically to damage the bacterial cell wall (Safdar *et al*., 2017; Weerasinghe *et al*., 2025).

CONCLUSION

The discovered *Acantella* sp. was characterized by a red, shrub-like branched body shape with a prickly and rough surface texture. Yield of *Acanthella* sp. e extract from the extraction used methanol solvents 3.22%, ethyl acetate 1.53%, and hexane 0.93%. Methanol extracted almost all components from the sponge cell matrix because of its polar properties. The

Acanthella sp. extracts demonstrated antibacterial activity against the pathogenic bacterium *E. coli*, where the methanol, ethyl acetate, and hexane extracts produced inhibition zones of 5.59 mm, 7.60 mm, and 5.90 mm. The ethyl acetate solvent dissolved components containing purer and more concentrated active compounds. Extract of *Acanthella* sp. with ethyl acetate solvent can be used as antibacterial candidates against the pathogenic bacteria *E. coli.*

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